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Studies on the Sensitivities of Esophageal Cancer to Anticancer Agents and the Supplementary Chemotherapy Combined with Surgical Treatment

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Introduction

Recently, surgical treatment of esophageal cancer has remarkably progressed by the development of pre-, mid-, and postoperative managements, but the curability of this cancer is much worse than those of cancers of the other gastrointestinal tract[21,32,39,43,44]. The rate of five-year survival in resected cases of esophageal cancer was reported to be 12 per cent by SATO et al., 16.1 per cent by NABEYA and 17.3 per cent by GUNNLAUGSSON[13,32,43]. Most causes of death are local recurrence or metastases towards the lymph nodes and other organs. Therefore, for the purpose of improving this rate, surgical operation of esophageal cancer should be combined with other supplementary therapies, such as radiotheraphy, chemotheraphy and immunotherapy. A marked effect of Bleomycin (BLM) or Peplomycin (PEP) has been observed on esophageal cancer and the effects of these drugs on cancer lesions are influenced by their concentrations and durations in the lesions[10,21,44,57,64]. The purpose of this study is to investigate the sensitivities of esophageal cancer to BLM and PEP, to establish the method to administer the effective and the smallest doses of these drugs for a long period and prevent BLM or PEP-induced pulmonary complications, and to improve the remote results of operation for esophageal cancer.

Chapter I. Sensitivity test of esophageal cancer to anticancer agents.

The procedure for determination of the sensitivities to anticancer agents.

In these studies, the sensitivities of esophageal cancer to anticancer agents were investigated by INAS (Inhibition of Nucleic Acid Synthesis) method by HIGASHI using ^{3}H-thymidine as a labeled precursor (Fig. 1)[15,16]. Relatively uniform and not necrotic tumor tissues were obtained from the fresh surgical specimens and sliced to about 500 μ in thickness. These tissue slices weighing 200 mg were preincubated with anticancer agents and TC 199 contained 20% calf serum for 4 hours in the flasks of Warburg's manometer, and followed by another one hour incubation with ^{3}H-thymidine of 6 μCi/ml as a labeled precursor. They were stirred and agitated with

Key words: Bleomycin, Peplomycin, Esophageal cancer, Sensitivity test, Supplementary chemotherapy.
索引語：ブレオマイシン，ペプロマイシン，食道癌，感受性試験，補助化学療法.
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Surgical specimen
↓ sliced with Stadie-Rigg's slicer to 500 μ in thickness
Tumor slices 200 mg
TC 199 contained 20% calf serum 1.8 ml
Anti-cancer agent solution 0.5 ml
↓ incubated for 4 hours at 37.5°C
0.5 ml of labeled precursor added
↓ incubated for 1 hour at 37.5°C
DNA extracted
Specific radioactivity estimated

Fig. 1. Procedure for determination of sensitivity. (INAS method by HIGASHI)

Warburg's manometer and incubated at 37.5°C. After the incubation for 5 hours, tumor tissues were taken out. DNA was extracted from these tumor tissues by the modified Schmidt-Thannhauser's method and its specific radioactivity was determined with liquid scintillation spectrometer (packard 3385). The quantity of DNA was determined by diphenylamine method. Thereafter, the percent radioactivity, that is, the ability of DNA synthesis was determined by the following expression[33,38]:

$$\text{Percent radioactivity (cpm/}\mu\text{g DNA)} = \frac{\text{Radioactivity of DNA (cpm/ml)}}{\text{Quantity of DNA (}\mu\text{g/ml)}}$$

BLM of 25, 5, 1 and 0.2 μg/ml were used in these experiments and all of them with the control were duplicated. HIGASHI et al. reported, that they decided to be sensitive in the cases that DNA synthesis of the tumor was inhibited below 70% in comparison with the control and this standard value agreed with the results from clinical practices[15,16]. In the present studies, the author decided to be sensitive to anticancer agents in the cases that DNA synthesis of the tumor was inhibited below 70% in comparison with the control.

The sensitivity tests of esophageal cancer to BLM were investigated in 48 cases from which esophageal cancer were resected in our clinic from March, 1979 to July, 1981 and the following items were analysed and discussed. The following descriptions conform to the descriptive rules of Japanese Society of Esophageal Diseases[26].

(1) Results of sensitivity tests analysed according to the locations of lesions.

In these clinical cases, the histology in three cases of the cardia (C) and one case of the abdominal esophagus (Ea) was adenocarcinoma and the other was squamous cell carcinoma. Thirty-one cases out of 48 cases, 65%, were sensitive to BLM.

Among the sensitivity rates analysed according to the locations of lesions, no noticeable difference was found (Table 1).

(2) Results of sensitivity tests analysed according to the types of roentgenological findings.

The superficial and tumorous types showed high sensitivity rates and the noticeable differences were found as compared with the serrated, funnelled and spiral types ($P=0.01$, Table 2).

(3) Results of sensitivity tests analysed according to the types of macroscopical findings.

In 2 cases out of 48 cases testing the sensitivity to anticancer agents, bypass operations were performed. In these cases, the resected metastatic lymph nodes were used for sensitivity tests.

Table 1. Results of sensitivity tests analysed according to the locations of lesions.

Location of lesion	Sensitivity (+)	Sensitivity (−)	Total
Ce	3	2	5
Iu	4	2	6
Im	18	7	25
Ei	5	3	8
Ea	0	1	1
C	1	2	3
Total	31	17	48

In 46 cases except these 2 cases, the results of sensitivity tests were analysed according to the types of macroscopical findings. The protruded and superficial types showed high sensitivity rates and the noticeable differences were found as compared with the ulcerative type (P=0.04, Table 3).

(4) Results of sensitivity tests analysed according to the types of histological findings.

Twenty-one cases out of 27 cases, 78%, which were classified into the well differentiated squamous cell carcinoma, were sensitive to BLM, and they showed high sensitivity rates and the noticeable differences were found as compared with the types of the moderately and poorly differentiated squamous cell carcinoma and adenocarcinoma (P=0.01, Table 4).

Table 2. Results of sensitivity tests analysed according to the types of roentgenological findings.

X-ray types	Sensitivity (+)	Sensitivity (−)	Total
Superficial	3	0	3
Tumorous	10 (13)	1 (1)	11 (14)
Serrated	1	4	5
Funnelled	0 (18)	2 (16)	2 (34)
Spiral	17	10	27
Total	31	17	48

(P=0.01, Fischer's exact test)

Table 3. Results of sensitivity tests analysed according to the types of macroscopical findings.

Macroscopical types	Sensitivity (+)	Sensitivity (−)	Total
Protruded	7	1	8
Superficial	3 (10)	0 (1)	3 (11)
Ulcerative	20	15	35
Total	30	16	46

(P=0.04, Fischer's exact test)

Table 4. Results of sensitivity tests analysed according to the types of histological findings.

Histological types	Sensitivity (+)	Sensitivity (−)	Total
Well different	21	6	27
Moderately different	5	6	11
Poorly different	4 (10)	2 (11)	6 (21)
Adenocarcinoma	1	3	4
Total	31	17	48

(P=0.01, Fischer's exact test)

(5) Results of sensitivity tests analysed according to the performance of preoperative radiotheraphy or chemotherapy by means of BLM.

The cases who hadn't undergone the preoperative treatment by radiotherapy or chemotherapy by means of BLM showed high sensitivity rates as compared with the cases who had undergone the treatment with irradiation above 3000 rads or the administration of BLM above 60 mg, preoperatively (Table 5, 6).

Regarding the results of sensitivity tests analysed according to the depth of invasion, lymph node metastasis and the existence of vascular invasion, there were no noticeable differences (Table 7, 8, 9).

Short summary: From these results, the following conclusions were obtained. Judging from the results of these sensitivity tests, BLM will be expected to be effective in the cases whose roentgenological type is superficial or tumorous, whose macroscopical type is protruded or superficial, and whose histological type is classified into the well differentiated squamous cell carcinoma, and on whom no preoperative treatments with irradiation or BLM has been performed.

(6) Differences between the sensitivities of main lesions and metastatic lymph nodes and between those of the central and peripheral portions of the tumor.

Materials and methods: The metastatic lymph nodes together with the main lesions in the clinical cases of esophageal cancer were used in these experiments, and the above-mentioned

Table 5. Results of sensitivity tests analysed according to the performance of preoperative radiotherapy.

Dose (rads)	Sensitivity (+)	Sensitivity (−)	Total
0	26	13	39
～3000	1	1	2
3000～	4	3	7
Total	31	17	48

Table 6. Results of sensitivity tests analysed according to the performance of preoperative chemotherapy by means of BLM.

Dose (mg)	Sensitivity (+)	Sensitivity (−)	Total
0	24	13	37
～60	4	1	5
60～	3	3	6
Total	31	17	48

Table 7. Results of sensitivity tests analysed according to the depth of cancer invasion.

Depth of invasion	Sensitivity		Total
	(+)	(−)	
m, sm	4	1	5
mp	5	2	7
a_1	5	2	7
a_2	9	3	12
a_3	7	8	15
Total	30	16	46

Table 8. Results of sensitivity tests analysed according to the histological stages of lymph node metastasis.

Lymph node metastasis	Sensitivity		Total
	(+)	(−)	
n_0	10	2	12
n_1	0	4	4
n_2	8	6	14
n_3, n_4	12	4	16
Total	30	16	46

sensitivity tests were performed. Thereafter, the differences between the sensitivities of main lesions and metastatic lymph nodes were compared and analysed. In the next experiments, the resected tumors were divided into the central and peripheral portions and the above-mentioned sensitivity tests were also performed. Thereafter, the differences between the sensitivities of the central and peripheral portions of the tumor were compared and analysed. In these experiments, the differences of sensitivities were compared at the level of BLM concentration of 5 μg/ml.

Results: The marked inhibition of DNA synthesis, that is, the high sensitivity to BLM was observed with the metastatic lymph nodes, and there was a noticeable difference as compared with main lesions ($P<0.05$, Fig. 2). Considering the merit of chemotherapy as a systemic treatment opposite to radiotherapy as a local treatment, this result was thought to be significant.

The marked inhibition of DNA synthesis, that is, the high sensitivity to BLM was observed

Table 9. Results of sensitivity tests analysed according to the existence of vascular invasion.

Vascular invasion	Sensitivity		Total
	(+)	(−)	
(+)	19	9	28
(−)	11	7	18
Total	30	16	46

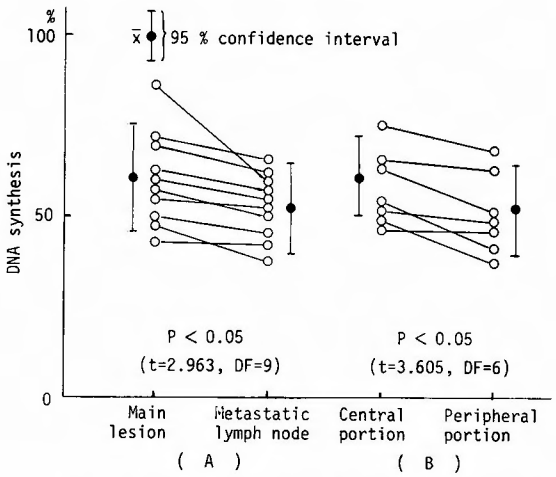

Fig. 2. Differences between the sensitivities of main lesions and metastatic lymph nodes (A) and between those of central and peripheral portions of the tumor (B).

with the peripheral portions of the tumor, and there was a noticeable difference as compared with the central portions of the tumor ($P<0.05$, Fig. 2). It was supposed that one of the causes to bring on this result was that DNA synthesis of cancer cells were more vigorous in the peripheral portions than the central portions.

(7) Differences of the sensitivities according to the performance of previous hyperthermic management (42°C).

Materials and methods: In the above-mentioned sensitivity test, previous hyperthermic management of the tumor tissue was performed with the method that tumor tissues were incubated at 42°C for the first one hour and followed by further incubation at 37.5°C. In the cases without previous hyperthermic management, whole incubation was performed at 37.5°C. With these two methods of incubation, the sensitivity tests of esophageal cancer to BLM were performed. Thereafter, the differences of the sensitivities in these two groups according to the performance of previous hyperthermic management (42°C) were compared and analysed. In these experiments, the differences were compared at the level of BLM concentration of 5 μg/ml.

Results: The tumor tissues which had undergone previous hyperthermic management showed a high sensitivity and a noticeable difference was observed as compared with those without this management ($P<0.05$, Fig. 3). From this result, the usefulness of hyperthermic chemotherapy for esophageal cancer was suggested.

(8) Difference between the sensitivities to BLM and PEP.

Materials and methods: Using the same and the half doses of PEP as compared with those of BLM, the above-mentioned sensitivity tests in the clinical cases of esophageal cancer were performed, and the difference between the sensitivities to BLM and PEP was compared and analysed.

Results: In the cases in which PEP were used at the same dose as that of BLM, PEP showed a marked inhibition of DNA synthesis and a noticeable difference was observed as compared

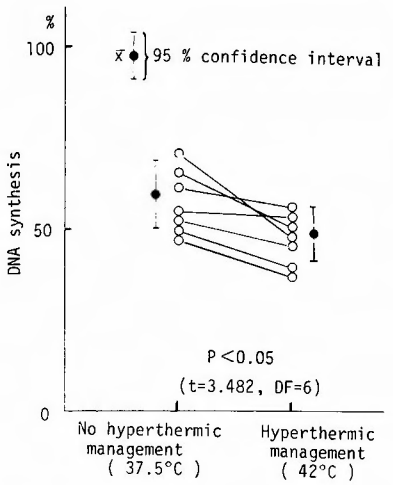

Fig. 3. Differences of the sensitivities according to the effect of previous hyperthermic management.

with BLM ($P<0.05$, Fig. 4). In the cases in which PEP was used at the dose half as much as that of BLM, PEP showed an equivalent inhibition of DNA synthesis as compared with BLM (Fig. 4). From these results, it was thought that PEP at the half dose brought on the same grade of anticancer effect as BLM.

Chapter II. Most proper administration dose of BLM, considering the sensitivity of esophageal cancer to anticancer agents.

(1) Necessary concentration of BLM for sensitive cases, determined by sensitivity tests.

As above-mentioned, the standard value of inhibition of DNA synthesis in the cases which were sensitive to BLM was decided to be above 70%. Therefore, the necessary concentrations of BLM for the main lesions in the sensitive cases were about 0.6 to 4 μg/ml (Fig. 5), and those for the metastatic lymph nodes were about 0.3 to 3 μg/ml (Fig. 6).

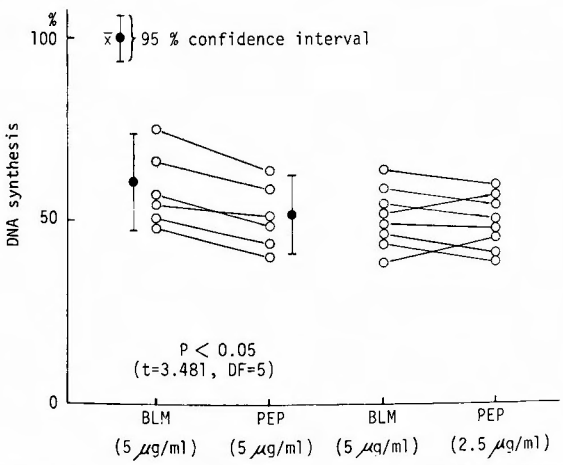

Fig. 4. Difference between the sensitivities to BLM and PEP.

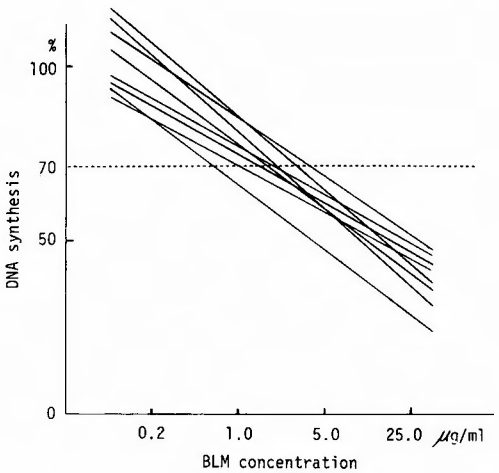

Fig. 5. Sensitivity curves of main lesions in cancer patients who showed the sensitivity to BLM

(2) BLM levels in the esophagus and lymph nodes in the clinical cases of esophageal cancer.

In 1975 SHIBATA measured BLM levels in the esophagus and lymph nodes in esophageal cancer patients following the intravenous injection[46].

Materials and methods: BLM was injected into 12 preoperative cases of esophageal cancer at a dose of 7.5, 10 and 15 mg, respectively. The drug levels in main cancer lesions and regional lymph nodes were measured 25 to 150 minutes following intravenous injection by bioassay, the Band Culture method by OKUBO[40].

Results: BLM concentrations were not in proportion to the administration doses. Those in main cancer lesions were 0.84 to 3.0 μg/ml and those in regional lymph nodes were 0.96 to 3.18 μg/ml (Table 10).

Short summary: From these results, the administration dose of BLM for esophageal cancer,

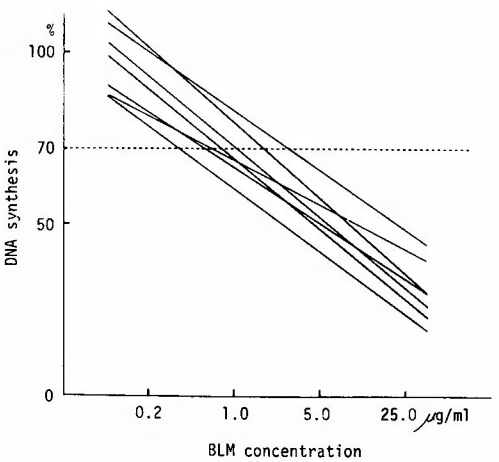

Fig. 6. Sensitivity curves of metastatic lymph nodes in cancer patients who showed the sensitivity to BLM

Table 10. Bleomycin levels in the esophagus and lymph nodes in cancer patients ($\mu g/g$)

Cases			Inj. dose of BLM (mg) i.v.	Cancer lesion	Lymph nodes	Histological types
No.	age	sex				
1	55	♂	7.5	2.16	3.18	moderately different
2	58	♀		1.92	—	well different
3	59	♂	10	3.0	3.0	poorly different
4	65	♀		1.72	—	well different
5	69	♀		1.93	—	well different
6	50	♀		1.89	—	adenocarcinoma
7	72	♀		2.88	—	adenocarcinoma
8	61	♂	15	1.89	1.78	well different
9	68	♂		2.01	1.71	well different
10	69	♂		1.1	1.1	moderately different
11	45	♂		1.05	0.96	well different
12	69	♀		0.84	—	well different

considering the sensitivity to anticancer agents, was thought to be almost adequate at the ordinary administration doses of BLM, ranging from 5 to 15 mg.

Chapter III. Comparison of various administration methods of BLM.

In these experiments, the superiority or inferiority of various administration methods of BLM were investigated by measuring the concentrations in blood and organs such as esophagus and lung etc. in dogs following BLM administration and the preventing effect of BLM on the development of experimental N-butyl-N-nitrosourethan (BNUR) esophageal cancer in rats.

The measurement method of the concentration of BLM.

In these experiments, the concentrations of BLM in blood and organs were determined by bioassay, the Band Culture method by OKUBO[40]. The strain of test organism: Bacillus subtilis PCI-219. Number of organisms: 2.1×10^7/ml. Medium: Müller-Hinton medium. Incubation time (37°C): 5 to 7 hours. Minimum inhibition concentration: 0.25 μg/ml[40,46].

Blood was centrifuged at 2000 rpm for 5 minutes, and its supernatant fluid was used for the measurement. In order to determine BLM concentration in the organs, the animals were sacrificed by exsanguination, their resected tissues were homogenized into an emulsion and diluted with the physiological saline solution twice the volume as these of the homogenates. These homogenates were kept in a refrigerator at 4°C for 24 hours. These supernatant fluids were used for the measurement of BLM concentration.

(1) BLM levels in blood and organs such as esophagus and lung etc. following various types of administration.

Materials and methods: Adult mongrel dogs weighing 7 to 12 kg were anesthetized by intramuscular injection of ketamine chloride at a dose of 10 mg/kg and the respiration was controlled by intratracheal intubation with a respirator. The femoral vein was cannulated by a "cutdown" tube to collect the femoral vein blood. Thereafter, the dog was injected with a BLM

solution, its concentration in the femoral vein blood was measured during 2 hours following injection and those in the esophagus, lung and others were measured.

The types of BLM administration were one shot intramuscular administration in a large dose of 1 mg/kg and 3 times divided intramuscular administration every 6 hours at the one time dose of 0.33 mg/kg. In these groups, BLM levels in blood and organs such as the esophagus and lung etc. were compared. The total doses of BLM administration were 1 mg/kg and the same in these groups.

Results: In the group of one shot administration in a large dose of BLM, the drug concentration in the femoral vein blood amounted to a peak level 30 minutes after the injection and then decreased gradually. In the group of divided administration of small doses, it amounted to a peak level 15 minutes after the injection and then decreased gradually. BLM concentration in blood was higher in the former group than the latter (Fig. 7). BLM concentrations in various organs 2 hours after intramuscular injection are shown in Table 11. BLM levels in the esophagus showed almost the same value in the both groups, but those in the lung showed a lower value in divided administration of small doses than one shot administration in a large dose and there was a noticeable difference between them ($P<0.01$, Table 11).

From these results, considering the pulmonary complication induced by BLM, the divided administration of small doses was thought to be more superior than one shot administration in a large dose.

(2) The effect of BLM on the development of experimental BNUR esophageal cancer in rats.

Materials and methods: In the first place, neoplasmas of the esophagus and the forestomach of rats were produced by the administration of carcinogens. According to TAKEUCHI's report in 1974, the experimental animals were Donryu rats and the carcinogen was BNUR[52]. In these experiments, sixty Donryu rats were administered with BNUR in drinking water at the

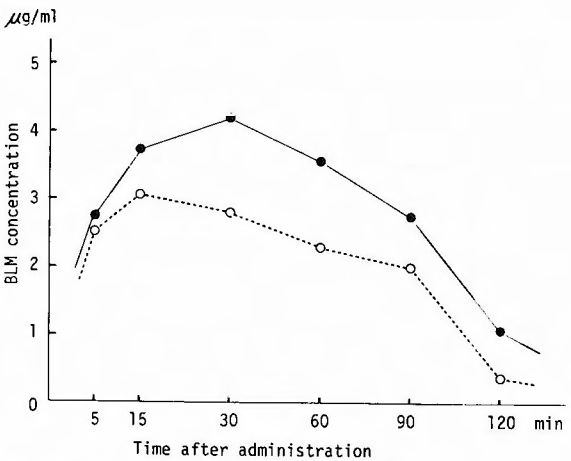

Fig. 7. BLM levels in blood following the intramuscular injection
●—● BLM 1 mg/kg one shot administration
○····○ BLM 0.33 mg/kg 3 times divided administration

Table 11. Bleomycin levels in various organs 2 hours after the intramuscular injection ($\mu g/g$)

Organs	Types of administration	
	BLM 1 mg/kg one shot administration	BLM 0.33 mg/kg divided administration (3 times)
Esophagus (mucous membrane)	6.2	6.0
Lung	3.4	2.3
Liver	2.4	1.9
Stomach	0	0

(t=5.069, DF=8; P<0.01)

concentration of 400 ppm. Twenty-nine weeks after the administration of BNUR, rats continued to receive the intramuscular injections of BLM by the following methods for 6 weeks. ① One shot administration in a large dose of BLM. Dose: 5 mg/kg. Number: one time per week. Methods and period: intramuscular injections for 6 weeks. The control group received the intramuscular injections of physiological saline solution by the same method. ② Divided administration of small doses of BLM. Dose: 1 mg/kg. Number: five times per week. Methods and period: intramuscular injections for 6 weeks. The control group received the intramuscular injections of physiological saline solution by the same method. In these groups with various types of BLM administration, the weight of the esophagus and stomach together with tumors, the number of neoplasmas of the esophagus, the occurrence rate of carcinoma in the esophagus and the histological effects of BLM were compared and analysed.

Results: Most rats in the group which received a divided administration of small doses of BLM had a small neoplasma of the forestomach and a small number of the polyp of the esophagus

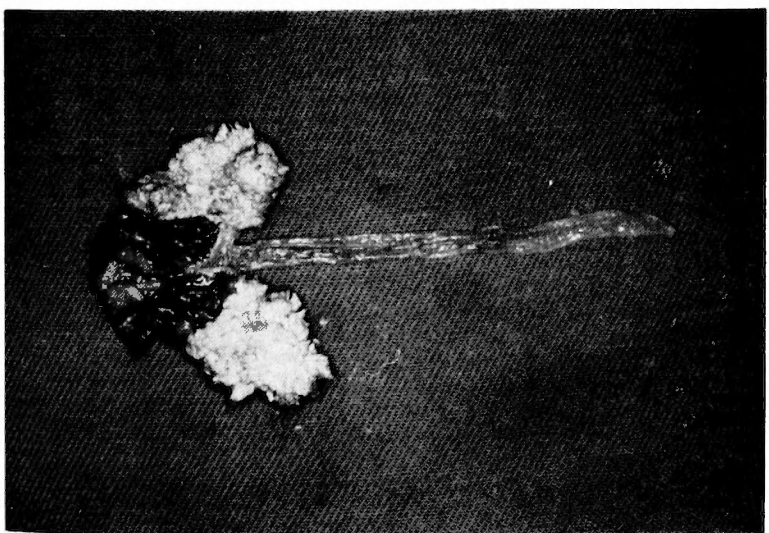

Photo. 1 BNUR-induced neoplasmas of the esophagus and the forestomach of rat Divided administration in small doses of BLM.

Photo. 2 BNUR-induced neoplasmas of the esophagus and the forestomach of rat
One shot administration in a large dose of BLM

(Photo. 1). Most rats in the group which received one shot administration in large dose of BLM had a large neoplasma of the forestomach and a large number of the polyp of the esophagus (Photo. 2). Most rats in the both control groups had a very large neoplasma of the forestomach and the esophagus (Photo. 3).

The weight of the esophagus and stomach together with tumors was lighter in the group which received a divided administration of small doses than one shot administration in a large dose and there was a noticeable difference ($P<0.01$, Table 12). The number of the polyp of the

Photo. 3 BNUR-induced neoplasmas of the esophagus and the forestomach of rat
No administration of BLM (control)

Table 12. Weight of esophagus and stomach together with tumors

	Types of administration			
	One shot in a large dose	Control	Divided administration in small doses	Control
Mean value (g)	8.67	10.43	4.90	9.65
Standard deviation	1.385	2.722	1.049	2.136
Standard error	0.524	0.962	0.396	0.756

(t=5.741, DF=12; P<0.01)

Table 13. Number of BNUR-induced neoplasmas of the esophagus of the rats

Number of neoplasmas	Types of administration			
	One shot in a large dose	Control	Divided administration in small doses	Control
~ 5	0	1	5	0
6~15	4	6	1	5
16~	3	1	1	3
Total	7	8	7	8

(t=2.818. DF=12; P<0.05)

esophagus was also smaller in the group which received a divided administration of small doses than one shot administration in a large dose and there was a noticeable difference (P<0.05, Table 13). Furthermore, according to the histological findings, the occurrence rate of carcinoma in the esophagus of rats was lower in the group which received a divided administration of small

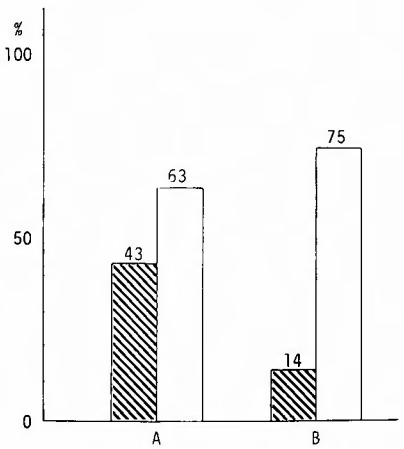

Fig. 8. Occurrence rate of carcinoma in the esophagus of rats
A) One shot administration in a large dose of BLM
B) Divided administration in small doses of BLM
□ Controls

doses than one shot administration in a large dose (Fig. 8).

From these results, the divided administration of small doses was thought to be more superior than one shot administration in a large dose as a type of BLM administration.

Even in the group which received a divided administration of small doses of BLM, the light microscopic observation showed that BLM was not so effective on the BNUR-induced squamous cell carcinoma, although the vacuolation and pyknosis of the nucleus and eosinophilic changes of the cancer cells were observed in several places[62].

Discussion

The effectiveness of preoperative radiation therapy on esophageal cancer has been reported by many authors[9,11,14,21,44,57]. CLIFFTON reported that the rate of resectability of esophageal cancer improved remarkably from 38% to 56% by this preoperative radiation therapy[7]. NAKAYAMA reported that the five-year survival rate of esophageal cancer was improved by this method[35]. AKAKURA reported that the rate of curative resection of esophageal cancer was improved remarkably from 25.8% to 67.6% by this method[2]. The radiation therapy and surgical operation for esophageal cancer, however, are classified into local treatment, and their effects may be obtained only on local lesion. On the other hand, chemotherapy and immunotherapy are general treatments whose effects may be obtained on both primary lesions of esophageal cancer and their remote lymphatic metastases. Recenty, chemotherapy has been combined with radiotherapy as the surgical adjuvant treatment of esophageal cancer.

BLM, which was discovered by UMEZAWA et al. in 1966, showed the marked effects on squamous cell carcinoma and lymphogenous tumors, such as Hodgkin's disease of Burkitt's tumor[3,19,20,47,54,55]. BLM is an anticancer antibiotics isolated from Streptomyces verticilus, and is a water-soluble and basic glycopeptide. The action of BLM inhibits DNA synthesis of the tumor and its effect is influenced by its concentration and duration in the lesion. Main side effects of BLM are fever, anorexia and pulmonary complications, especially pulmonary fibrosis, which is most troublesome side effect for the operation of esophageal cancer. Recently, new bleomycin derivative, peplomycin (PEP) has been developed. It has been reported that this drug has few side effects, such as pulmonary complications, as compared with BLM[45,48]. Tumor cells tend to converge, enzymatically, to common tissue cells. Therefore, from the enzymatical viewpoint, so far as anticancer drugs act only upon the enzyme system related to the autonomic atypical multiplication of the tumor, its effective action cannot be expected without some side effects[25]. Therefore, when BLM and PEP are administered for esophageal cancer, the method to administer continuously an effective and smallest doses of these drugs for a long period, as to prevent the pulmonary complications, must be established. For this purpose, the author performed the sensitivity test of esophageal cancer to BLM and PEP in the first place.

Recently, various sensitivity tests to anticancer agents have been developed. Among the methods based on the change in the activity of various enzymes, there are SDI test (Succinic Dehydrogenase Inhibition test), CAP method (Cell-Agar-Plate method), APD test (Acid Phos-

phatase Deviation test) and INK method, and besides, BLACK and DIPAOLO have reported the method of this type[5,8,22,28,36,61]. Among the methods which used a tissue culture, there are reports of WRIGHT, HURLEY and TAGUCHI[17,51,59]. Among the methods which used living bodies, there are reports of ICHIHASHI, TANI and TAGUCHI[18,50,53]. Among the methods based on the inhibition of DNA synthesis, there are reports of BICKIS, VOLM, WOLBERG, WÜST and FUJIMOTO, and INAS method (Inhibition of Nucleic Acid Synthesis method) by HIGASHI et al. is classified into this category[4,12,15,16,56,58,60]. From the results of sensitivity tests of esophageal cancer to BLM and PEP which the author performed in this experiment, it was made clear that these drugs showed a high sensitivity rates in the cases, who showed the superficial or tumorous type in roentgenological findings, the protruded or superficial type in macroscopical findings and well differentiated squamous cell carcinoma in histological findings, and who had undergone no preoperative treatments with radiation or BLM. Therefore, in the cases of esophageal cancer who have some of these findings, it is thought that the effects of the chemotherapy with BLM and PEP are to be expected. Therefore, the importance of BLM and PEP as a chemotherapeutic agent in systemic treatment was emphasized. CASPERSSON et al. classified human cancer cells into A cell, such as the cells in the peripheral portions of cancer lesions, around blood vessels or invading into neighbouring tissues, which had an active synthesis of protein and therefore was rich in the volume of nucleic acid, and B cell[6]. As the cause that the peripheral portions of tumor showed a higher sensitivity to BLM than the central portions of tumor, it is supposed that the former have more A cells, which have an active DNA synthesis, than the latter. Recently, the effectiveness of hyperthermic chemotherapy on malignant tumors has been reported[1,29,30,37,41]. SUGIMACHI et al. has performed hyper-thermo-chemo-radiotherapy with BLM on esophageal cancers and has reported that this method has been useful[49]. In our experimental results in vitro, the usefulness of hyperthermic chemotherapy was suggested. Therefore, it is thought that this is a practicable and effective enough method of treatment and for example, hyperthermic management using double balloon catheter which YASUMOTO of our clinic has developed, should be promising[63]. Recently, BLM derivative, PEP has been developed. It has been reported that this drug has a larger effect and less side effects than BLM[45,48,64]. From the results in the present study considering the sensitivity tests to anticancer agents, it was thought that PEP at the half dose brought on the same grade of anticancer effect as BLM. Besides, considering the side effects of this drug, this may be a useful drug henceforth as an anticancer agent for esophageal cancer. As above-mentioned, the administration dose of BLM for esophageal cancer, considering the sensitivity to anticancer agents, was thought to be almost adequate at the smallest among the doses of 5 to 15 mg which have been administered ordinarily at present. However, in order to increase the effect of BLM, it is necessary to keep the concentration of BLM at high levels. Therefore, in selecting the methods of BLM administration, we had better put emphasis on local administration in addition to systemic. Local administrations of BLM for esophageal cancer have been performed using various methods, that is, intramural injection into the esophageal wall, local administration using spongel-BLM during operation, continuous infusion into the posterior mediastinum, selective intraarterial infusion by Seldinger's method, local administration

into the lumen of the esophagus using double balloon catheter and infusion into the thoracic duct[23,27,31,34,63]. Oil-BLM remains in the injected tissue for a long period, so it may be more effective than Solution-BLM as local chemotherapy[42]. Taking into consideration the concentrations in blood and organs such as the esophagus and lung etc. in dogs following BLM administration, and the preventing effect of BLM on the development of experimental BNUR esophageal cancer in rats, the divided administration in small doses was thought to have less side effects and larger effect and to be a more superior method of BLM administration than the one shot administration in a large dose. In our clinic at present, the sensitivity tests to BLM and PEP are performed on the resected tissues of esophageal cancer, and the plan of postoperative treatments are decided conforming to these results[24].

Conclusion

For the purpose of establishing the standard method of BLM and PEP administrations as supplementary chemotherapy combined with surgical treatment for esophageal cancer, the author examined the sensitivities of esophageal cancer to these drugs by INAS method using ^{3}H-thymidine as a labeled precursor, and determined the concentrations of anticancer agents in cancer lesions by the Band Culture method by OKUBO. On the other hand, the author investigated the superiority or inferiority of various methods of BLM administration by measuring BLM concentrations in organs such as the esophagus and lung etc., and by observing the preventing effect of BLM on the development of experimental BNUR esophageal cancer in rats. The results were as follows:

1) Thirty-one cases out of 48 cases of human esophageal cancer, 65%, were sensitive to BLM. As to the types of roentgenological findings, the superficial and tumorous types showed a higher sensitivity rate than the serrated, funnelled and spiral types ($P=0.01$). As to the types of macroscopical findings, the protruded and superficial types showed a higher sensitivity rate than the ulcerative type ($P=0.04$). As to the types of histological findings, the well differentiated squamous cell carcinoma showed a higher sensitivity rate than the moderately and poorly differentiated squamous cell carcinomas and adenocarcinoma ($P=0.01$). The cases who hadn't undergone preoperative treatments with radiation and BLM showed also a higher sensitivity rate than the cases who had undergone the treatments with radiation above 3000 rads or BLM above 60 mg.

2) The sensitivities were higher in metastatic lymph nodes than main lesions and in the peripheral portions of tumor than the central portions of it ($P<0.05$, $P<0.05$).

3) The tumor tissues which had undergone previous hyperthermic management (at 42°C during one hour) showed a higher sensitivity than those which hadn't undergone this management ($P<0.05$). The usefulness of hyperthermic chemotherapy was suggested.

4) PEP at the half dose brought on the same grade of anticancer effect as BLM.

5) Necessary concentrations of BLM in main lesions and metastatic lymph nodes in the sensitive cases determined by sensitivity tests were 0.6 to 4 and 0.3 to 3 μg/ml, respectively. On

the other hand, BLM concentrations in main lesions and lymph node metastases following the intravenous injection at a dose of 7.5, 10 and 15 mg were 0.84 to 3.0 and 0.96 to 3.18 μg/ml, respectively, and were not in proportion to the administration doses. From these results, the administration dose of BLM for esophageal cancer was thought to be almost adequate at the smallest among the doses of 5 to 15 mg which are administered ordinarily at present.

6) BLM concentrations in the mucous membrane of esophagus in dogs following the one shot administration in a large dose and the divided administration of small doses showed almost the same value in the both groups, but those in the lung showed a lower value in the latter than the former ($P<0.01$). Besides in the rats administered with BNUR, the weight of the esophagus and stomach together with tumors, the number of the polyp of esophagus and the occurrence rate of carcinoma in the esophagus following one shot administration of BLM in a large dose and its divided administration of small dose, were lower in the latter than the former. From these results, considering the pulmonary complications, an important side effect induced by BLM and the effects of treatment by BLM, the divided administration of small doses was thought to be more superior than the one shot administration in a large dose as the methods of BLM administration.

From the above-mentioned results, although the sensitivity of esophageal cancer to BLM and PEP had an individual difference in the clinical cases, the administration dose of BLM for the sensitive cases of esophageal cancer was thought to be almost adequate at the smallest among the doses which are administered ordinarily at present and, considering the pulmonary complications and the effects of treatment, the divided administration of small doses was more superior than the one shot administration in a large dose as the methods of BLM administration.

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和文抄録

食道癌の制癌剤感受性と手術補助制癌剤投与法に関する研究

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食道癌手術補助療法としての BLM および PEP の至適投与法の基準を確立するために，食道癌に対する BLM および PEP の感受性を ^{3}H-thymidine を labeled precursor とする INAS 法で，また制癌剤病巣内分布量を帯培養法で測定する一方，BLM 各投与法の優劣をイヌ食道，肺などの臓器内濃度，ラットにおける BNUR 実験食道癌発生に対する BLM 投与の抑制効果などから検討し，次のような成績を得た．

1) ヒト食道癌48例中31例，65%に BLM 感受性を認めた．X線型では，表在型および腫瘤型は鋸歯型，漏斗型およびらせん型に比べて，肉眼型では，隆起型および表層型は潰瘍型に比べて，また組織型では，高分化型扁平上皮癌は中分化型および低分化型扁平上皮癌や腺癌に比べて感受性率が高かった．また術前非照射群および術前 BLM 非投与群は術前 3,000 rads 以上照射群および術前 BLM 60 mg 以上投与群より感受性率が高かった．

2) リンパ節転移は主癌病巣より，また癌腫の辺縁部は中心部より感受性が高かった．

3) 温熱処理（42°C, 1時間）を加えた癌組織は無処理 (37.5°C) の癌組織に比べて感受性が高く，hyperthermic chemotherapy の有用性が示唆された．

4) PEP は BLM の半量でほぼ同程度の制癌作用を示した．

5) 感受性試験による感受性症例の主癌病巣およびリンパ節転移の BLM 必要濃度はそれぞれ 0.6～4 および 0.3～3 μg/ml であり，一方ヒト食道癌における BLM 各 7.5, 10, 15 mg 静注後の主癌病巣およびリンパ節内濃度は投与量と関係なく，それぞれ 0.84～3.0 および 0.96～3.18 μg/ml であった．これらの結果より食道癌に対する BLM 投与量としては，現在一般的に行われている 5～15 mg の投与量の最小量である程度十分であると考えられた．

6) イヌを用いた BLM 大量衝撃投与および小量分割投与後の食道粘膜内濃度は両群ほぼ同じ値であったが，肺では小量分割投与の方が低く，また BNUR 投与ラットを用いた BLM 大量衝撃投与と小量分割投与における腫瘤を含めた食道および胃の重量，食道のポリープ発生数および食道における発癌率はいずれも小量分割投与群で低かった．これらの結果より BLM の重要な副作用である肺合併症を考慮し，また BLM の治療効果からみて，BLM の投与法としては小量分割投与の方が大量衝撃投与よりすぐれていると考えられた．

以上の成績から，食道癌に対する BLM および PEP の感受性には症例による個人差があるが，BLM を食道癌手術補助療法として使用する際，感受性症例では現在一般的に行われている投与量のうちの最小量で十分であり，投与法としては副作用および治療効果の点からみて，小量分割投与の方が大量衝撃投与よりすぐれていることを明らかにした．